PRELIMINARY STUDIES ON THE DISEASES OF LARVÆ OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWM.)

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Early in investigations on the Japanese beetle (*Popillia japonica* Newm.) it was found that many of the larvæ, both in the field and in storage cellars, became diseased and soon died. Disease became so rampant that sometimes fully 50 per cent of the larvæ collected and stored for biological studies and parasite rearing died. Besides causing loss to experimental work, it was soon realized, when systematic surveys of the larval population in the field were undertaken, that disease might be at least partially responsible for the observed variations in numbers of the insect in the infested area.

For the past seven years, at least, the Japanese beetle has been decreasing in abundance in the older infested area centering around Riverton, N. J., where it was first found in this country. This decrease has taken place when the insect has not only been abundant, but increasing in numbers in more recently infested areas. Entomologists working with the insects have put forth several theories to explain the reduction in the older area. It is now well established that shortage of rainfall at the times when eggs and small larvæ predominate in the soil causes a reduction in larval population. However, shortages of rainfall alone can not explain the general trend occurring during the entire period of reduction. Other possible agencies, such as birds, predatory and parasitic insects, soil treatments to destroy the larvæ, and spraying operations to kill the adults, contribute to this reduction to a a minor degree.

PREVIOUS STUDIES

The earliest studies on the diseases of Japanese beetle larvæ were made by the late George E. Spencer, at the Japanese beetle laboratory of the Bureau of Entomology, from 1926 to 1927. When healthy larvæ were inoculated by the puncture method with pure bacterial cultures isolated from diseased larvæ, it was plainly shown that some of the organisms were highly pathogenic. In some preliminary experiments Spencer attempted to determine the methods of infection under normal field conditions, and found that injured larvæ acquire the disease more readily than normal ones.

It has already been stated that a high mortality often occurs among larvæ stored in a cool place for winter experimental work. Such larvæ are placed in large tubs with soil. Larvæ crowded thus often bite or nip one another, and wounds so produced may conceivably aid in the spread of disease from a few affected individuals in storage containers to healthy larvæ near-by. If disease were due mainly to crowding, it would be expected to be more prevalent in fields having a high larval population, but from our recent field surveys there is no clear evidence that this is the case.

Spencer found that larvæ are attacked to some extent by fungi. Though relatively scarce under field conditions, fungus-infected larvæ have at times been commonly found in experimental rearing cages. Through the efforts of Dr. L. O. Howard, two cultures of *Isaria densa* Auct., a pathogen of *Melolontha,* were obtained from France. When 94 healthy Japanese beetle larvæ were placed in soil contaminated with this fungus, a fungous growth developed on 48 of them. Of 12 larvæ placed in pure cultures of the fungus, 5 became diseased.

In 1928 studies on the diseases of larvæ were renewed at the Japanese beetle laboratory by Dr. Henry Fox, the work being undertaken in cooperation with Dr. R. W. Glaser of the New Jersey Department of Agriculture. Bacterial cultures from black or brown diseased larvæ were obtained and used in field treatments. The treated plots were frequently examined and no diseased larvæ were found. As this work was getting under way, a promising parasitic nematode, *Neoaplectana glaseri* Steiner, was encountered, on which attention was thereafter concentrated.

PRESENT STUDIES

In September 1933 studies on the diseases of the larvæ were renewed at the Japanese beetle laboratory in cooperation with the junior author.

Diseases of the Larvæ

The sick and the dead larvæ taken in the field were classified tentatively into three groups, the black group, the white group, and the fungous group. Preliminary studies on these groups indicated that there are more than three diseases affecting the larvæ.

Black group.—Dead larvæ the remains of which become black, dark gray, or brown constitute the majority of the diseased grubs found. The remains of larvæ of this group were crushed and suspended in distilled water. This suspension was strained and used to inoculate healthy larvæ by puncture, by dipping the larvæ, by treating the food of larvæ, and by treating blotting paper with which the tin rearing containers were lined. The resulting mortalities were but little higher than those found in untreated checks. In a series of tests, however, in which a diseased larva was placed in a tin salve box with a healthy one, there resulted a mortality of 40 per cent at the end of 3 weeks. Two checks showed mortalities of 4 and 8 per cent, respectively.

While microscopic examinations of dead larvæ of this group showed numerous bacteria, only a few species occurred on agar plates inoculated with material from the decaying remains. Among these were spore-bearing species forming colonies having an opaque frosted appearance, non-spore-producing species forming gray colonies, and others forming bluish ones.

Pure cultures of three of these species were used for inoculating healthy larvæ by the puncture method.[1] Larvæ used for checks were punctured with a sterile needle. All larvæ were kept singly in sterilized tin salve boxes lined with moistened blotting paper. Wheat was added as food. Of the 10 larvæ in each of the three series, 5, 7, and 8, respectively, were dead after 2 weeks. All check larvæ were alive. The diseased larvæ ex-

[1] A. R. Rolfs, of the Japanese beetle laboratory, made these inoculations and ably assisted in carrying out these tests.

hibited a variety of symptoms. In one series the 5 larvæ that died turned dark while sick and after death became jet black. The head was discolored, the body wall was flabby, and there was no odor. In the second series the 7 larvæ that died became whitish gray at first and later turned black. The head was not discolored and there was no odor. The discoloration appeared first on the thorax at the point of puncture. Some black watery fecal matter exuded from the anus. In the third series the larvæ that died showed a grayish discoloration at first, black patches then appeared, and the entire body became a dull black within a week after death. Each of the three cultures showed considerable virulence. All the larvæ that died finally became black, though there was some variation in the earlier symptoms. Although the different symptoms noted for this group may be due to the presence of different species of bacteria during decay of the remains, it seems quite as probable that more than one disease may be present in this group.

White group.—At times larvæ that have an unnatural milky-white appearance are found in the field. These are frequently alive when encountered. Tissues escaping from the torn body wall of larvæ sick or recently dead of this disease are filled with a microorganism in pure or nearly pure culture. Attempts to grow this species on the usual as well as special media have not yet been successful. It seems likely that an infectious disease is present in this group and that the organism present in large numbers is the exciting cause.

Fungous group.—Larvæ have been found which bear tufts of fungous growth along their sides, invading the tissues and extending outward through the sclerotized integument. These larvæ may be alive when found. Other larvæ were dead when found, and these larvæ were firm, brittle, and invaded with mycelial growth. Probably more than one disease is present in this group.

Studies to Determine the Cause of Mortality in Field and Greenhouse Plots

On the laboratory grounds at Moorestown, N. J., many field-plot experiments have been carried on. In some of the plots, largely

checks in soil-treatment experiments, there have been attempts in recent years to increase the normal larval population by adding larvæ to these plots, but such attempts have often been unsuccessful, as the larvæ died rapidly.

In an effort to explain this situation samples of soil were gathered from several sources. One sample was taken from a plot on the laboratory grounds where larvæ had failed to develop, a second sample from a bed in a rose greenhouse in which larvæ had shown a high mortality, a third sample from a field in northern New Jersey beyond the present range of the Japanese beetle, a fourth sample from a local field and disinfected by heating, and a fifth sample from the same field as the fourth but untreated. Six-ounce tins were sterilized and fitted with wire-screen partitions to divide each tin into four compartments. In each test 25 such tins were filled with the soil to be tested and a healthy larva was placed in each compartment. The mortality for each soil tested is based, therefore, on 100 larvæ. Wheat was added for food. These tests were started on December 5, 1933, and continued for 44 days before the final check up was made.

The mortality was 14 per cent in the soil from the field plot where larvæ failed to survive, 65 per cent in soil from the rose house, 15 per cent in soil obtained outside the infested area, 15 per cent in the disinfected field soil, and 16 per cent in untreated field soil. The mortalities were surprisingly close in all the soils except that from the rose house. In this sample some of the dead larvæ showed a fungous growth, while others turned a chocolate brown, being flabby at first and later watery, and disintegrating rapidly with an unpleasant odor. The organisms causing these disease conditions have not been determined.

Incidence of Disease in Infested Areas

During 1933 and 1934 larval surveys in grass sod were made weekly at eight stations in the older infested area under the direction of Dr. Henry Fox. The percentage of diseased larvæ found in the soil population is given in Table 1.

At some of these stations the percentage of diseased larvæ was unusually high, while at others great fluctuations occurred from month to month. The data show, however, that the incidence of

Table 1.—Percentage of diseased Japanese beetle larvæ found in larval surveys made at eight stations in New Jersey and Pennsylvania, 1933 and 1934

Month	Diseased larvæ	
	1933 Per cent	1934 Per cent
January	0.0	1.7
February	0.2	no record
March	1.3	1.4
April	2.2	1.4
May	5.4	1.8
June	10.2	1.0
July	0.4	0.1
August	0.5	1.5
September	1.6	3.1
October	3.0	.....
November	2.2	.....
December	2.8	.....

disease is relatively low in July, when the old brood is scarce in the soil and the new brood consists largely of eggs and newly hatched larvæ. There is a gradual increase during the summer, fall, and spring, the high point being reached in May or June, when the larvæ are mature. In June 1933, 10 per cent of all larvæ found were diseased. At one station this month 29 per cent were dead or dying from disease. It is also evident from Table 1 that diseased larvæ formed a much larger proportion of the soil population in 1933 than they did in 1934. The reason for this difference is not now apparent. The data show further that the reduction in the larval population between the fall of 1933, as indicated by the average of all surveys made in September and October, and the early part of June 1934 was only 17 per cent, whereas the average reduction for this period during the past seven years, as determined by Dr. Fox, has been 34 per cent. It is impossible to draw definite conclusions from two years' data, but from the evidence at hand it would seem that the diseases of the larvæ, by their greater prevalence some years than others, may be largely responsible for the fluctuation of the beetle population from year to year.

Larval surveys show that disease is now present in some degree in most of the area infested by the Japanese beetle. In the fall of 1933 and the spring of 1934 diseased larvæ were found in considerable numbers at certain points in Delaware, New Jersey, and Pennsylvania where beetles have been abundant for only a few years. A study of the survey data from 27 stations widely scattered in New Jersey and Pennsylvania in the spring of 1934 shows no consistent relationship between the extent of disease and the age of an infestation or the density of the larval population. At these 27 stations from 0 to 12 per cent of the larval population were diseased at the time the surveys were made.

FURTHER STUDY PLANNED

The two outstanding problems relative to the larval diseases at the present time are (1) to devise means by which larvæ for biological study and for parasite rearing may be protected from infection and (2) to determine whether the diseases of this insect can be used in its control. Both these problems need to be thoroughly investigated. This is almost an untouched field for study. On July 1, 1934, under a cooperative arrangement between the New Jersey Agricultural Experiment Station and the Japanese beetle laboratory of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, a thorough study of the entire disease problem, both in the laboratory and in the field, was begun.

SUMMARY

Diseases cause a high mortality among Japanese beetle larvæ. Among larvæ used for experimental work this mortality sometimes reaches 50 per cent.

For purposes of the present paper these diseases are classified from gross appearance of the affected larvæ into three groups—the black group, the white group, and the fungous group. The majority of the dead larvæ found in the field belong to the black group. The cause of the disease or diseases of this group is not known. Probably only one disease is present among larvæ of the white group. The tissues of the sick and dead larvæ of this group are filled with a microorganism, in pure or nearly pure culture, which is probably the causal agent of the disease. The

infectiousness of the disease or diseases of the larvæ in the fungous group has not yet been determined.

In an effort to explain the high larval mortality in field plots at the Moorestown laboratory in recent years, larvæ were placed in soil from several sources. It was found that the mortality was nearly the same in soil from the laboratory grounds where larvæ had failed to develop, in soil from beyond the present infested area, in locally gathered field soil disinfected by heating, and in soil from the same source as the last but untreated. In soil from a rose greenhouse the mortality was much higher. Many of the larvæ from this source showed evidence of disease.

The disease among larvæ in the present infested area varies greatly from year to year and at different times during the year. Disease is usually highest in May or June just before pupation and lowest when the new brood appears in July. Larval surveys in 1933 and 1934 show no consistent relationship between the extent of disease and the age of infestation or density of the larval population.